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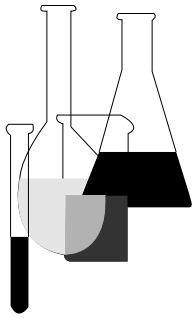
Ecological Effects Test Guidelines

OPPTS 850.3030 Honey Bee Toxicity of Residues on Foliage



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

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OPPTS 850.3030 Honey bee toxicity of residues on foliage.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*).

(2) **Background.** The source material used in developing this test guideline is OPP 141–2 Honey Bee—Toxicity of Residues on Foliage (Pesticide Assessment Guidelines, Subdivision L—Hazard Evaluation; Nontarget Insects) EPA report 540/09-82-019, 1982.

(b) **Purpose.** This guideline is designed to develop data on the residual toxicity to honey bees of chemical substances subject to environmental effects test regulations. The Agency will use these and other data to assess acute hazards to bees.

(c) **Definitions.** The definitions in section 3 of the Toxic Substances Control Act (TSCA) and 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline. In addition, the following definitions apply to this guideline:

Test substance is the specific form of a chemical or mixture of chemicals that is used to develop the data. For the purposes of this test, test substance is a representative end-use product.

(d) **Test procedures**—(1) **Summary of test.** (i) Test bees may be obtained directly from hives or from frames kept in an incubator. The test substance is applied to the test crop (alfalfa is preferred) at typical label rate(s). At predetermined posttreatment time intervals, treated foliage is harvested and test bees are caged on the foliage and on control (untreated) foliage. Bees are monitored and observed for mortality and signs of intoxication during the exposure period. Mortality is determined after 24 h of exposure to the treated foliage.

(ii) A test is unacceptable if more than 20 percent of the control bees die during the test.

(2) **Definitive test**—(i) **Pesticide application and harvest of foliage.** A single application of the test substance will be made to each of nine alfalfa plots. Plots should be at least 1 m_2, in alfalfa grown according to standard agricultural practices. Applications should be made with a hand sprayer. Three control plots should be maintained and treated identically to the treatment plots, with the exception of pesticide application. After residues have aged for the appropriate time period, alfalfa foliage will be harvested from the test plots and returned to the laboratory.

(ii) **Exposure to test substance.** On the day of test initiation, young bees should be collected from the incubator or directly from the hive, immobilized with CO_2 or N_2, and placed in holding cages. To initiate the test, harvested foliage is chopped, mixed, and divided into 15–g portions.

Each portion is placed into a single test chamber. Bees in the holding cages are again immobilized, and introduced into the test chambers until each chamber contains at least 25 bees.

(iii) **Controls.** (A) A negative (untreated) control is required during the test. Control plots should be treated identically to treatment plots, except for pesticide application. Control and test bees should be kept under the same environmental conditions.

(B) A concurrent positive control with a substance of known toxicity is not required. However, a quarterly or semiannual test with a laboratory standard (reference toxicant) is recommended as a means of detecting possible interlaboratory or temporal variation. A laboratory standard is also recommended when there is any significant change in source of bees.

(iv) **Number of animals tested.** Six replicates should be assigned to each treatment and control group, with a minimum of 25 bees for each replicate.

(v) **Dosages and posttreatment intervals.** At a minimum, the test substance should be evaluated at the maximum label rate. Multiples of the maximum rate may be evaluated if desired. Residues should be allowed to weather in the field for a specific time period prior to collection of foliage for testing. For purposes of comparison, test samples could be collected 3, 8, and 24 h after application. If mortality rates of bees exposed to 24–h–old residues is greater than 25 percent, sampling at 24–h intervals should continue until mortality of bees exposed to the treated foliage is not significantly greater than control mortality.

(vi) **Duration of test.** The definitive test consists of the exposure of bees to the treated foliage followed by an observation period of 24 h.

(vii) **Observations.** (A) Environmental conditions following pesticide application will be monitored at the field site. Environmental information to be collected will include temperature, precipitation, relative humidity, wind speed, and estimated cloud cover.

(B) Bees should be observed for mortality and toxicological responses at least once within the first 4 h after exposure and at test termination. Prior to the evaluation at test termination, observations should be made without disturbing or removing bees from the test chambers; for these observations, estimates of mortality and effects are sufficient. Dead bees should not be removed from the test chambers until the test is terminated.

(C) Throughout the test period, all signs of intoxication, other abnormal behavior, and mortality should be recorded and reported by dosage level and by time of occurrence. Signs of intoxication are those behaviors apparently due to the test chemical and may include a wide variety of behaviors, such as ataxia, lethargy, and hypersensitivity. All signs of in-

toxication and any other abnormal behavior, that may or may not be attributed to the test substance, should be reported.

(e) **Test conditions**—(1) **Test species**—(i) **Selection.** (A) Honey bee, *Apis mellifera*, is the test species. Bees may be obtained from on-site colonies or from a commercial apiary. All control and treatment bees used in a test should be from the same source.

(B) Bees used in the test should be in apparent good health. Only bees from disease-free colonies should be used, and they should be kept in conditions conforming to proper cultural practices.

(C) Test should be conducted on worker bees 1 to 7 days old at test initiation. No acclimation period is necessary. Bees used in the test should be assigned randomly to treatments and controls.

(D) During holding and testing, bees should be shielded from excessive activity or other disturbance. Bees should be handled only as much as is necessary to conform to test procedures.

(ii) **Diet.** (A) A 50 percent sugar/water solution should be provided ad libitum throughout the holding and test periods. Purified or distilled water should be used for the sugar solution.

(2) **Facilities.** (i) Tests should be conducted indoors with bees being maintained in small test chambers. Test chambers may be constructed of metal, plastic, wire mesh, or cardboard, or a combination of these materials. Chambers must be constructed so that a vial containing sugar water may be attached.

(ii) Testing is done indoors to control lighting and other environmental variables. Temperature should be maintained between 25 and 35 °C, with relative humidity between 50 and 80 percent. It is recommended that test bees be maintained in the dark except during dosing and observations.

(f) **Reporting.** The report should include, but not necessarily be limited to, the following information:

(1) Name and address of the facility performing the study and the dates of the study.

(2) Objectives and procedures stated in the approved protocol, including any changes in the original protocol.

(3) The test and, if used, control substances identified by name, Chemical Abstracts Service (CAS) number or code number, source, lot or batch number, strength, purity, and composition or other appropriate characteristics.

(4) A description of the methods used, including:

(A) Description of housing conditions, including type, size, and material of pens, and the approximate test room temperature, humidity, and lighting.

(B) Methods of application, foliage collection, and placement of foliage in test chambers.

(C) Methods of assigning bees to test chambers.

(D) Frequency, duration, and methods of observations.

(5) A description of the test system used, including the scientific name of the test species, number used, condition, age at test initiation, and source of test bees.

(6) A description of the dosages, numbers of bees and replicates per dose, and method and time of administration. The reported results should include, for the definitive test, a description of signs of intoxication and other abnormal behavior, including time of onset, duration, severity, and number affected at each dose level and control.

(7) A description of all circumstances that may have affected the quality or integrity of the data.

(8) The name of the sponsor, study director, principal investigator, names of other scientists or professionals, and the names of all supervisory personnel involved in the study.

(9) The signed and dated reports of each of the individual scientists or other professionals involved in the study, including each person who, at the request or direction of the testing facility or sponsor, conducted an analysis or evaluation of data or specimens from the study after data generation was completed.

(10) The locations where all raw data and the final report are stored.

(11) The statement prepared and signed by the quality assurance unit.

(g) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Johansen, C. et al. Bee Research Investigations. Dept. of Entomology, Washington State University, unpublished, 22 pp. (1977).

(2) Lagier, R.F. et al. Adjuvants Decrease Insecticide Hazard to Honey Bees. College of Agriculture Research Center, Washington State University Bulletin 801, 7 pp. (1974).

(3) U.S. Environmental Protection Agency, Standard Evaluation Procedure, Honey Bee—Toxicity of Residues on Foliage, EPA Report No. 540/09–85–003 (1985).